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C2U
(71) Applicant
Roussel-Uclaf
35 Boulevard des
Invalides, 75007 Paris,
France
(72) Inventors
Roger Deraedt,
Vesperto Torelli,
Jean Vacher
(74) Agents
Sanderson & Co.
97, High Street
Colchester, Essex, CO1
1TH

(54) (20S)-3α-(Aminoacetylamino)-5α-pregnan-20-ol and its Acid Addition Salts

(57) The novel title compounds are prepared from funtumidine by *N*-acylation and salification, and are used in the treatment of auto-immunity diseases.

The chemical formulae appearing in the printed specification were submitted in formal form after the date of filing.

GB 2 057 448A

SPECIFICATION

Derivative of 5-α-pregnan-20-ol and its Acid Addition Salts

The present invention relates to a new derivative of 5α-pregnan-20-ol and its acid addition salts, as well as the preparation of this derivative and its salts, their use as medicaments and compositions containing the same.

According to the invention there is provided (20S)-3α-(aminoacetylamino)-5α-pregnan-20-ol or an acid addition salt thereof with an organic or a mineral acid.

As examples of acid addition salts there may be mentioned salts formed with hydrochloric, hydrobromic, hydroiodic, nitric, sulphuric, phosphoric, acetic, formic, benzoic, maleic, fumaric, succinic, tartaric, citric, oxalic, glyoxylic or aspartic acid, an alkanesulphonic acid such as methanesulphonic acid or an arylsulphonic acid such as benzenesulphonic acid.

(20S)-3α-(aminoacetylamino)-5α-pregnan-20-ol is a new compound which has never been described until now. (20S)-3α-(aminoacetylamino)-5α-pregnan-20-ol as well as its acid addition salts with pharmaceutically-acceptable acids shows interesting pharmacological properties as is illustrated by the results of tests given hereinafter in the experimental portion; these compounds, in particular, stimulate the action of the immune defence system in animals including man, potentiating especially the production of IgE (ivmunoglobulins E).

(20S)-3α-(aminoacetylamino)-5α-pregnan-20-ol as well as its acid addition salts with pharmaceutically-acceptable acids can, therefore, be used for the treatment of auto-immunity diseases resulting from a deficiency in certain lymphocytes, whether they be non-specific diseases of the connective tissue of a given organ such as, for example, rheumatoid arthritis or systemic lupus erythematosus, or whether they be specific diseases of an organ such as the thyroiditis pymphygus or haemolytic anaemia.

The compounds of the invention thus can be used as adjuvant treatment in antibiotherapy and in anti-cancer chemotherapy.

Accordingly, the invention includes the use of (20S)-3α-(aminoacetylamino)-5α-pregnan-20-ol as well as its acid addition salts with pharmaceutically-acceptable acids as a medicament in the above-mentioned treatments.

The invention also includes a pharmaceutical composition, which composition comprises, as active principle, at least one compound according to the invention, together with a pharmaceutically-acceptable excipient or carrier.

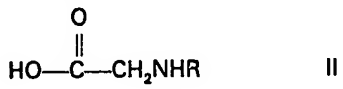
The compositions of the invention can be administered by a variety of routes, especially by an oral, rectal or parenteral route.

Moreover, the compositions may be solid or liquid and may be presented in a wide variety of pharmaceutical forms such as those currently used in human medicine, for example, plain or sugar-coated compressed tablets, gelatin capsules, granules, suppositories and injectable preparations, which may be prepared according to known methods.

The active principles or principles of the compositions may be mixed with such excipients as are usually employed in pharmaceutical compositions, and the excipient used may be solid or liquid as appropriate to the pharmaceutical form chosen. The excipient may be selected from a wide range of organic and inorganic solids, and aqueous and non-aqueous liquids, of which examples include talc, gum arabic, lactose, starch, magnesium stearate, fatty substances of animal or vegetable origin such as cocoa butter, paraffin derivatives or glycols. These excipients may be compounded with or there may be used alone one or more wetting, dispersing or emulsifying agents and/or one or more preservatives.

The dose administered may be varied according to the complaint treated, the person concerned, the route of administration and the compound used. Thus the dose may be, for example, of from about 1 to about 100 mg per day, when administered orally in an adult human being.

The invention also provides a process for preparing a compound according to the invention, which process comprises reacting funtumidine or (20S)-3α-amino-5α-pregnan-20-ol with an amino acid of the general formula:



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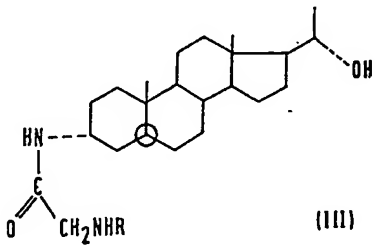
In which R represents as easily-cleavable protective group, especially one easily cleavable by hydrogenation, to obtain a compound of the general formula:



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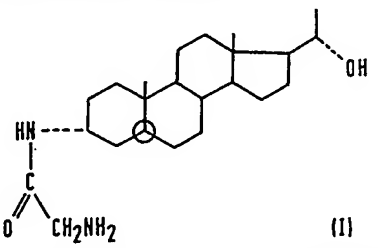
and subjecting the compound of formula III to the action of an agent for cleaving the group R, to obtain a compound of the formula:



(II)

which is subjected, if desired, to the action of an acid to form a salt thereof.

As an easily-cleavable protective group R there may be used, for example, the carbobenzyloxy group or the carbotert-butyloxy group. In an especially preferred process the protective groups R is the carbobenzyloxy group.

In the process of the invention the reaction between (20S)-3α-amino-5α-pregnan-20-ol and the amino acid of formula II preferably takes place in the presence of a condensation agent. The aim of the condensation agent, is of course, to activate the acid function of the amino acid of formula II. As condensation agent there may be used a carbodiimide of the general formula:

$$R_1—N=C=N—R_2$$

in which R_1 and R_2, which may be the same or different, represent an alkyl radical containing from 1 to 8 carbon atoms, optionally bearing a dialkylamino radical, or R_1 and R_2 represent a cycloalkyl radical.

As examples of the above condensation agents there may be mentioned, for example, dicyclohexylcarbodiimide or 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide of which the latter is a preferred agent.

As a condensation agent there may also be used an alkyl chloroformate such as, for example, methyl or ethyl chloroformate, as well as an alkyl pyrophosphite such as, for example, ethyl pyrophosphite.

As cleaving agent, it is preferred to use hydrogen in the presence of palladium.

The formation of salts can be effected according to standard techniques, for example, by the addition of the appropriate acid to the basic compound.

The starting material used in the process of the invention, namely (20S)-3α-amino-5α-pregnan-20-ol, is a known product (see Merck Index, 9th edition, heading 4144).

The following examples illustrate the invention without, however, limiting it.

Example 1:
(20S)-3α-(aminoacetylamino)-5α-pregnan-20-ol (Hydrochloride Salt and Base).

Stage A:
(20S)-3α-(benzyloxycarbonylaminoacetyl-amino)-5α-pregnan-20-ol

5.2 g of (20S)-3α-amino-5α-pregnan-20-ol and 5.2 g of N-carbobenzyloxyglycine were dissolved in 150 cc of chloroform and 15 cc of pyridine. The solution was agitated in an ice-bath under nitrogen and 3.9 g of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride were added. At the end of one hour there were then added 310 mg of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride, the agitation being maintained for half-an-hour in the ice-bath and then the mixture being diluted with a saturated solution of sodium acid carbonate. The insoluble portion was then filtered off, washed separately with a solution of sodium bicarbonate, with water, with normal hydrochloric acid and finally with water, then dried at 50°C. There were thus recovered 5.1 g of insoluble product.

The chloroform phase was separated from the filtrate, then washed with water, with normal hydrochloric acid and finally with water, dried and distilled to dryness. There were thus obtained 4.6 g of semi-crystalline residue. The insoluble product (5.1 g) and the semi-crystalline residue (4.6 g) were combined and made into a paste at reflux with 25 cc of methanol. The paste was then chilled, and the solid separated, washed with methanol and dried at 50°C to provide 7.05 g of the desired product, melting at 250 to 252°C. This product was used as such in the following stage, while an analytical sample obtained by recrystallisation from acetic acid had a melting point of 254°C.

Stage B:
(20S)-3α-(aminoacetylamino)-5α-pregnan-20-ol Hydrochloride.

7 g of the product obtained in Stage A were dissolved with heating in 150 cc of acetic acid. The solution thus obtained was agitated under nitrogen bubbled into it, allowed to cool to about 45°C and 700 mg of 10% palladium on charcoal were added thereto. The bubbling-in of nitrogen was then replaced by slight bubbling-in of hydrogen. At the end of one hour a current of nitrogen bubbled into it, allowed to cool to about hydrogen and the bubbling continued for 15 minutes. The catalyst was then filtered off and rinsed with acetic acid and the filtrate evaporated to dryness. The thus-obtained crystalline product was then dissolved in 50 cc of methanol and the desired salt precipitated by the addition of 4 cc of a 5.5 N solution of hydrochloric acid in ethanol.

The thus-obtained suspension was diluted with 50 cc of absolute ethanol, concentrated by half and left for one night at about +5°C. The solid product was then separated by filtration, washed with ethanol and dried, giving 4.43 g of the desired product.

The filtrate, mixed with 0.5 cc of a 5.5 N solution of hydrochloric acid in ethanol and concentrated to a small volume, provided a second yield of 0.35 g of the desired product. The pure product, melting at 271°C, was obtained after recrystallisation from 80% ethanol. $[\alpha]_D^{20}=+19.5\pm1°$ (1%, pyridine containing 10% of water).

Stage C:
Preparation of the Base

The crude hydrochloride obtained in Stage B was dissolved in hot water. By the addition of an excess of 2N sodium hydroxide the base was precipitated. This was separated, washed with water, dried and recrystallised from methanol.

The pure base thus-obtained had a melting point of 269°C and $[\alpha]_D^{20}=+25°\pm1°$ (1%, pyridine containing 10% of water).

Example 2:
Pharmacological Study of the Hydrochloride Salt of Example 1, Hereinafter Called Product A, using LEVAMISOLE as a Comparison Compound

LEVAMISOLE is a well-known compound (see Merck Index, 9th edition, heading 8949) which has immuno-regulating properties, see Cancer Research *35* 927 (1975) or New England Journal of Medicine *289* (21) 1148 (1973).

A—Potentiation of the Production of IgE

Female mice, weighing 28 to 30 g, were immunised by the sub-cutaneous route with ovalbumin mixed with alumina on days 0 and 14 and their serum sampled on day 21 to determine the IgE antibodies formed. The respective amounts of antigen (ovalbumin) and adjuvant (alumina) injected were selected so that the production of antibodies was minimal.

The compounds studied were administered by the subcutaneous route three hours before the first immunisation.

The determination of the IgE's was carried out by means of the passive skin anaphylaxia test. This test consists in causing in an animal, by intravenous administration of the antigen, an antigen-antibody reaction in a skin area where previously there have been injected antibodies prepared in another animal with the same antigen. This reaction is made visible due to a dye injected at the same time as the antigen: there is an appearance, at the point of injection of the antibodies, of a coloured spot, demonstrating the bursting of the sensitised cells and the increase in the capillary permeability resulting therefrom. The greatest dilution of the serum which gives a spot having a diameter of 11 to 13 mm in all the animals were investigated. The equivalent dilution of the serum was injected intradermally at a volume of 0.1 ml into male rats weighing, on average, 250 g, in the region of the back. Forty-eight hours later the animals receive intravenously 0.5 ml of a solution containing 0.5% of ovalbumin and 1% of Evans blue in an isotonic solution of sodium chloride. Thirty minutes after this injection they were sacrificed by bleeding and the diameter of the blue spot was measured on the turned-back skin.

The results obtained were as follows:

Product a	0.5 mg/kg sub-cutaneously
Levamisole	20 mg/kg sub-cutaneously

Conclusion: according to this test Product A is much more immunostimulating than Levamisole.

B—Chronic Arthritis Caused by the Adjuvant

The injection of Freund-type adjuvant (Mycobacterium butyricum at 6 mg/ml in Bayol 55) into a rear paw causes in the rat of primary inflammatory lesion then, after a period of latency of 13 to 15 days, the initiation of secondary inflammations affecting the non-injected rear paw as well as the front paws, the tail and the ears.

This secondary arthritis can be compared with human rheumatoid arthritis since the intervention of auto-immunity reactions is admitted among its determining factors.

Male rats, aged 42 to 50 days, received by intraplantar injection 0.10 ml of Freund adjuvant.

The treatment began on the day of the injection of the adjuvant and lasted until the sacrifice of the animals on the 17th day.

The criteria of estimation of the activity of the substances are, in general:

— the increase in weight of the animals, always restrained more or less proportionally to the intensity of the arthritis;

— the increase in volume of the injected and non-injected rear paws with reference to the average volume of the corresponding paws of the normal controls;

— the arthritis of the front paws, of which the too-small volume does not lend itself to plethysmometric measurement and is, therefore, marked subjectively from 0 to 3 according to the intensity of the inflammation;

— the arthritis of the ears and of the tail, marked 1 or 0 according to the presence or the absence of nodosities.

In the present test the effect on the secondary inflammations, that is to say on the arthritis of the non-injected rear paw, the arthritis of the front paws and the inflammations of the ears and of the tail, was observed in particular.

The active dose which reduced the secondary inflammations by at least 50% was investigated and the results were as follows:

Product A active dose 1 mg/kg (sub-cutaneously)

Levamisole active does 50 mg/kg (orally)

Conclusion: product A is, therefore, very active against the secondary phenomena of arthritis caused by the adjuvant whereas a very high subtoxic dose of Levamisole is necessary to establish a comparable effect.

Example 3:
Example of a Pharmaceutical Composition

Compressed tablets were prepared, corresponding to the following formula:

Hydrochloride of Example 1	10 mg
Excipient (talc, starch, magnesium stearate q.s. for	1 compressed tablet

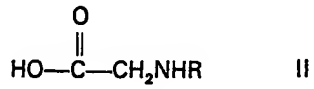
Claims

1. (20S)-3α-(aminoacetylamino)-5α-pregnan-20-ol or an acid addition salt thereof with an organic or a mineral acid.

2. A compound according to claim 1 in the form of a salt formed with hydrochloric, hydrobromic, hydroiodic, nitric, sulphuric, phosphoric, acetic, formic, benzoic, maleic, fumaric, succinic, tartaric, citric, oxalic, glyoxylic, aspartic, an alkanesulphonic or an arylsulphonic acid.

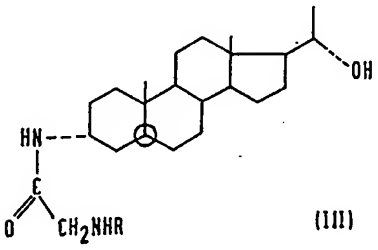
3. A process for preparing a compound as defined in claim 1, which process comprises

reacting (20S)-3α-amino-5α-pregnan-20-ol with an amino acid of the general formula:



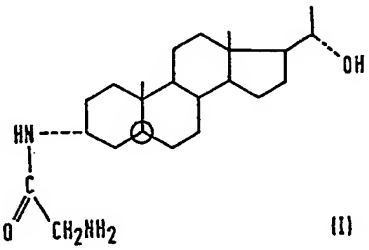
II

in which R represents an easily-cleavable protective group, to obtain a compound of the general formula:



(III)

and subjecting the compound of formula III to the action of an agent for cleaving the group R, to obtain a compound of the formula:



(II)

which is subjected, if desired, to the action of an acid to form a salt thereof.

4. A process according to claim 3, wherein the protective group is one cleavable by hydrogenation.

5. A process according to claim 4, wherein the protective group R is the carbobenzyloxy group.

6. A process according to any one of claims 3 to 5, wherein the reaction between (20S)-3α-amino-5α-pregnan-20-ol and the amino acid of formula II takes place in the presence of a condensation agent.

7. A process according to claim 6, wherein the condensation agent is a carbodiimide of the general formula:

$$R_1—N=C=N—R_2$$

in which R_1 and R_2, which may be the same or different, represents an alkyl radical containing from 1 to 8 carbon atoms, optionally bearing a dialkylamino radical, or R_1 and R_2 represent a cycloalkyl radical.

8. A process according to claim 7, wherein the carbodiimide used is 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide.

9. A process according to any one of claims 4 to 8, wherein the group R is cleaved using hydrogen in the presence of palladium.

10. A process according to claim 3 and substantially as hereinbefore described with reference to Example 1.

11. A compound as defined in claim 1 when prepared by a process according to any one of claims 3 to 10.

12. A pharmaceutical composition, which composition comprises, as active principle, at least one compound according to any one of claims 1, 2 or 11, together with a pharmaceutically-acceptable excipient or carrier.

13. A composition according to claim 12 substantially as hereinbefore described specifically.

14. A compound according to any one of claims 1, 2 or 11 or a composition according to claim 12 or claim 13 when used as a medicament in the treatment of auto-immunity diseases.

15. A compound according to any one of claims 1, 2 or 11 or a composition according to claim 12 or claim 13 when used as a medicament substantially as hereinbefore described specifically.

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